Map of the Human type IX collagen molecule showing the 3α chains which constitute the heteropolymer and their collagenous (COL) and non-collagenous (NC) domains.

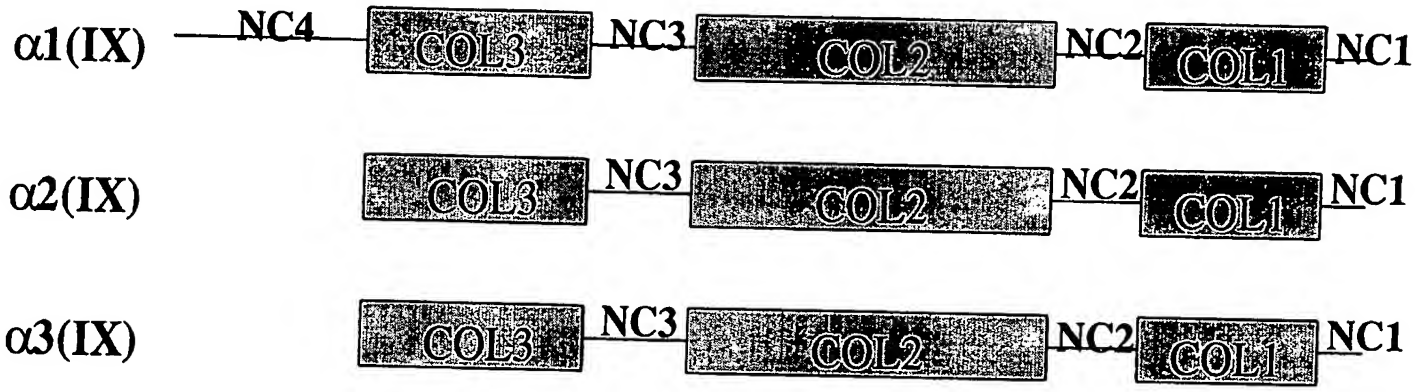


Figure 1

BEST AVAILABLE COPY

Figure 2

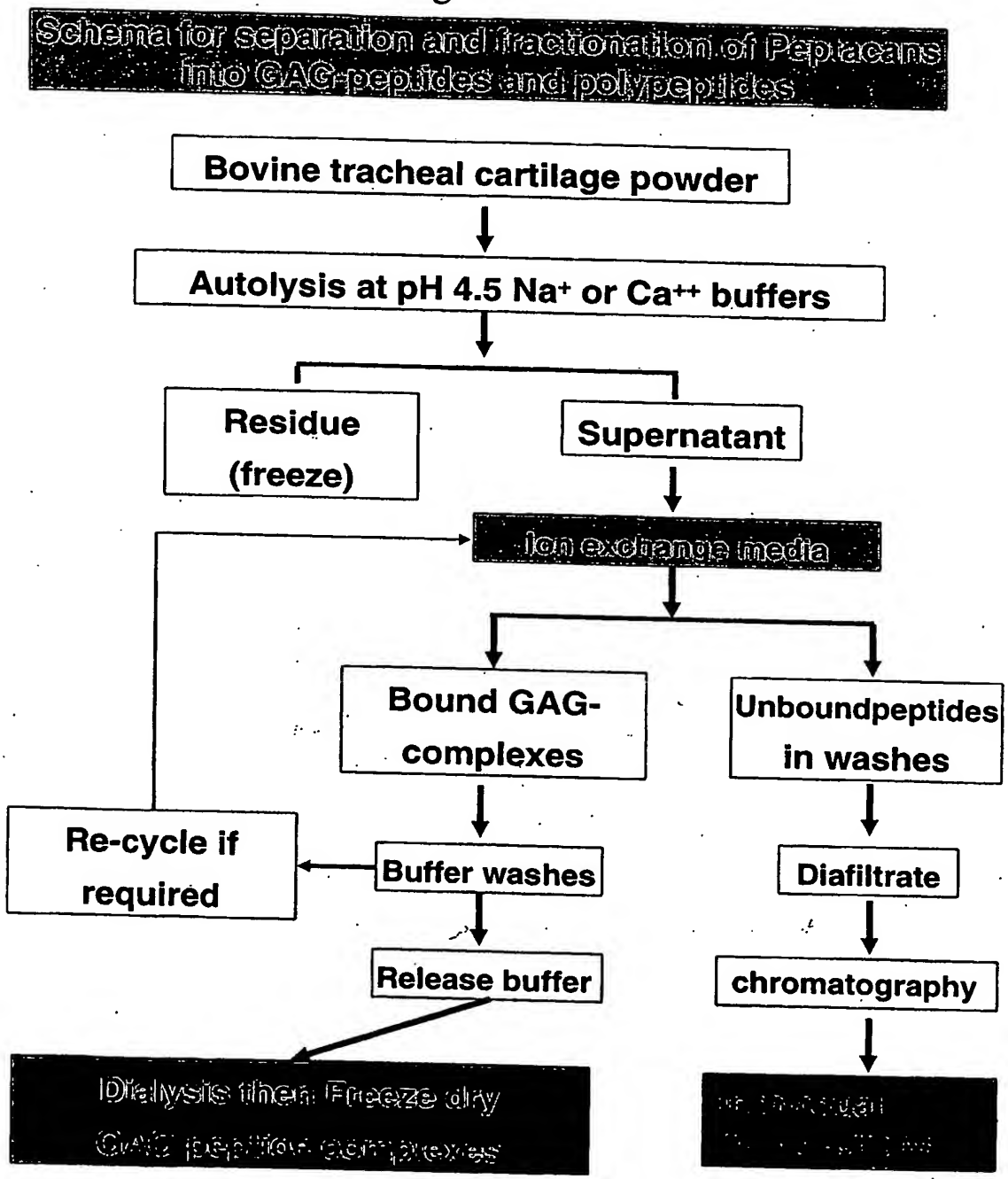

Schema for separation and fractionation of Peptacans into GAG-peptides and polypeptides
Bovine tracheal cartilage powder
Autolysis at pH 4.5 Na+ or Ca++ buffers
Residue (freeze)
Supernatant
ion exchange media
Bound GAG-complexes
Unboundpeptides in washes
Re-cycle if required
Buffer washes
Diafiltrate
Release buffer
chromatography
Dialysis then Freeze dry

Figure 3

SDS-PAGE of Proteins (polypeptides) Isolated from CaP

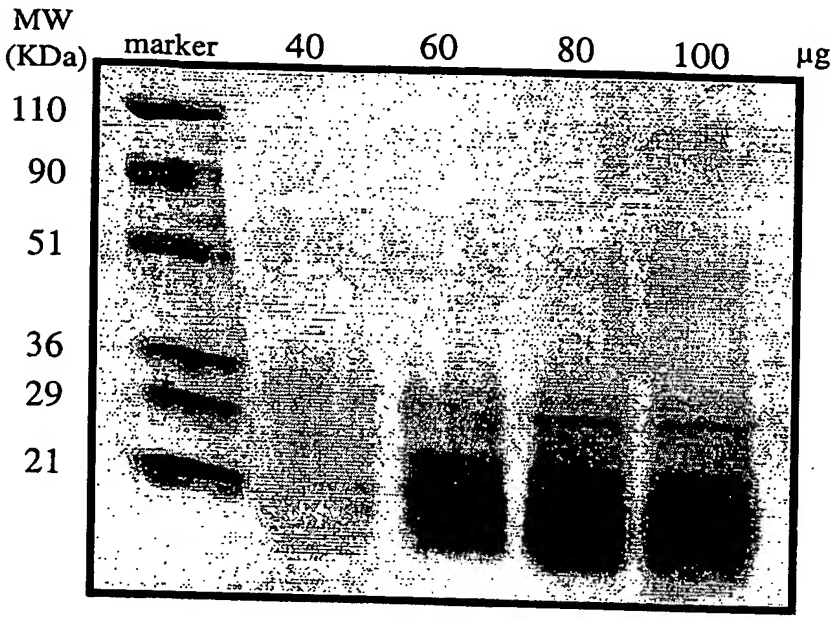
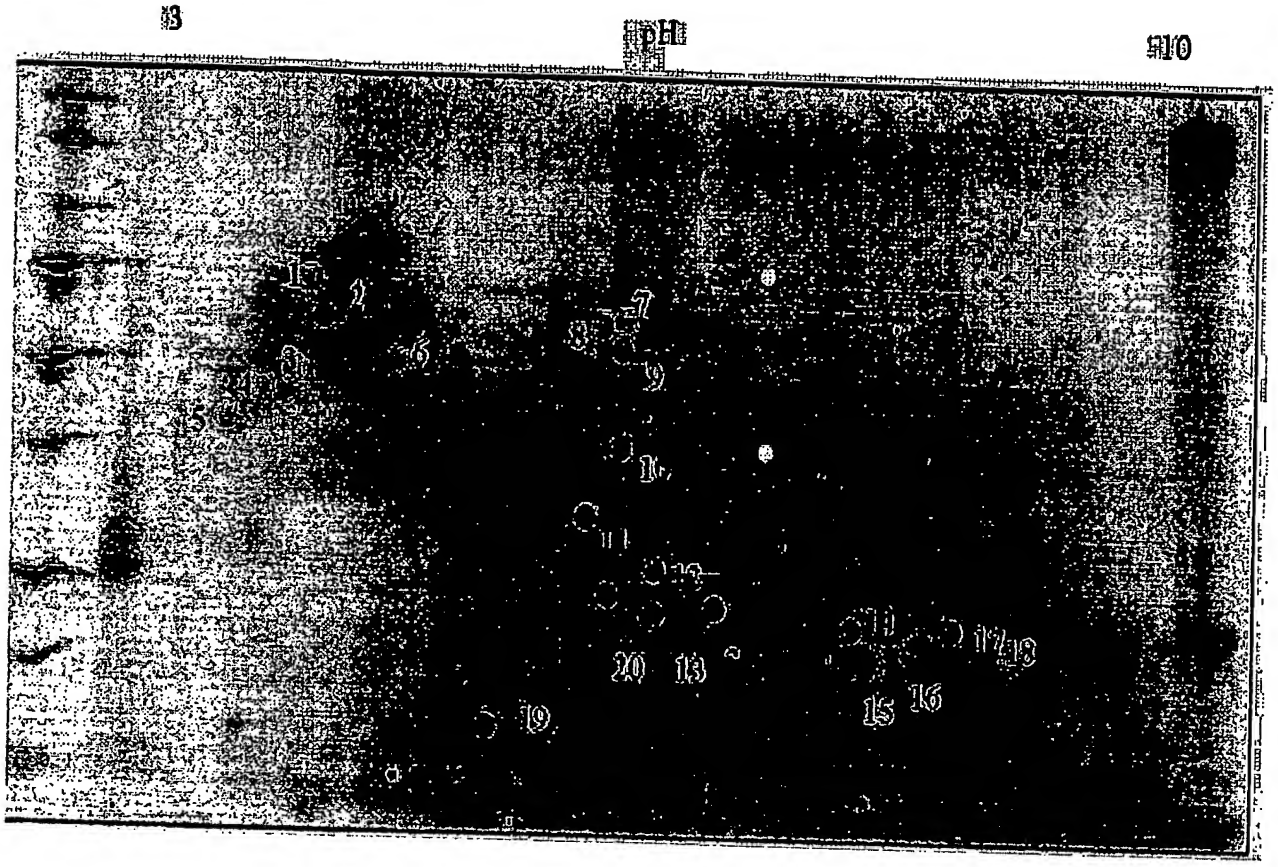


Figure 4



CBT protein sample, pH3-10 gradient. Proteins annotated for MALDI-MS

FIGURE 5

1
Tentative
NCF1_BOVIN
Neutrophil cytosol factor 1
Molecular weight: 45346
Matches: 5
MOWSE Score: 1.6076937e+003
Likelihood: 1.96e+003
Coverage: 14.80 %
Matching peptides:
MW Delta Start End Sequence
841.4657 -88.56 120 126 (K)VRPDD LK(L)
886.4760 -21.99 127 134 (K)LPTDS QVK(K)
886.4508 -50.37 283 291 (K)AGQDV AQAK(S)
* 1164.5822 0.02 328 336 (R)NSVRF MQQR(R)
1730.7934 -0.01 56 70 (K)EMFPI EAGDI NPENR(I)
1891.9197 23.26 170 188 (K)GSSSQ MALAT GDVVD VVEK(N)
OR
Tentative
ALBU_BOVIN
Bovine Serum Albumin
Molecular weight: 69294
Matches: 5
MOWSE Score: 7.6716406e+001
Likelihood: 1.88e+003
Coverage: 8.73 %
Matching peptides:
MW Delta Start End Sequence
711.3664 59.75 29 34 (K)SEIAH R(F)
* 959.5400 -35.02 210 218 (R)EKVLA SSAR(Q)
* 1000.5818 -23.99 233 241 (R)ALKAW SVAR(L)
1385.6133 -28.71 286 297 (K)YICDN QDTIS SK(L)
* 1961.9404 26.67 139 155 (K)LKPDP NTLCD EFKAD EK(K)
2

Tentative

Score: 0.19, 6 matching peptides: P35445 (COMP_BOVIN) pI: undefined, Mw: undefined

Cartilage oligomeric matrix protein (COMP) (Fragment). - Bos taurus (Bovine).

user mass matching [Delta] #MC modification positionpeptide
mass (ppm) mass
887.4908 887.4404 -56.88 0 Cys_PAM: 10 8-14 DNCPLVR
1181.5063 1181.4463 -50.84 0 2xCys_PAM 26-34 WGDACDNCR
1226.6927 1226.631 -50.35 1 Cys_PAM: 69 62-71 IRNPVDNCPK
1337.5306 1337.491 -29.64 0 Cys_PAM: 53 50-61 GDACDDDIDGDR
Page 3
1370.707 1370.6369 -51.19 0 168-179 LVPNPGQEDMDR
1386.6544 1386.6318 -16.32 0 MSO: 177 168-179 LVPNPGQEDMDR
11.4% of sequence covered:

3

No Good Match

4

No Good Match

5

No Good Match

6

No Good Match

7

ALBU_BOVIN
Bovine Serum Albumin
Molecular weight: 69294
Matches: 11
MOWSE Score: 9.5664269e+006
Likelihood: 4.27e+003
Coverage: 17.96 %
Matching peptides:
MW Delta Start End Sequence
926.4862 -137.94 161 167 (K)YLYEI AR(R)
1162.6234 -74.58 66 75 (K)LVNEL TEFAK(T)
1282.7033 -96.76 361 371 (R)HPEYA VSVLL R(L)

1304.7088 -90.40 402 412 (K)HLVDE PQNLI K(Q)
* 1438.8045 -82.02 360 371 (R)RHPEY AVSVL LR(L)
1478.7881 -92.19 421 433 (K)LGEYG FQNAL IVR(Y)
1510.8355 -83.71 438 451 (K)VPQVS TPTLV EVSR(S)
1566.7354 -87.40 347 359 (K)DAFLG SFLYE YSR(R)
* 1638.9304 -66.09 437 451 (R)KVPQV STPTL VEVSR(S)
1414.6802 -38.28 569 580 (K)TVMEN FVAFV DK(C)
+ Methionine Sulfoxide
1893.9294 -61.74 508 523 (R)RPCFS ALTPD ETYVP K(A)
+ Cysteine acrylamide
Page 4
8
ALBU_BOVIN
Bovine Serum Albumin
Molecular weight: 69294
Matches: 13
MOWSE Score: 7.6034479e+007
Likelihood: 1.17e+004
Coverage: 21.42 %
Matching peptides:
MW Delta Start End Sequence
926.4862 -72.22 161 167 (K)YLYEI AR(R)
1162.6234 -28.39 66 75 (K)LVNEL TEFAK(T)
1282.7033 -47.50 361 371 (R)HPEYA VSVLL R(L)
1304.7088 -31.77 402 412 (K)HLVDE PQNLI K(Q)
* 1438.8045 -39.49 360 371 (R)RHPEY AVSVL LR(L)
1478.7881 -32.08 421 433 (K)LGEYG FQNAL IVR(Y)
1510.8355 -19.52 438 451 (K)VPQVS TPTLV EVSR(S)
1518.7388 -25.79 139 151 (K)LKPDP NTLCD EFK(A)
1566.7354 -34.55 347 359 (K)DAFLG SFLYE YSR(R)
* 1638.9304 -19.36 437 451 (R)KVPQV STPTL VEVSR(S)
* 988.5488 24.47 221 228 (R)LRCAS IQK(F)
+ Cysteine acrylamide
1414.6802 8.58 569 580 (K)TVMEN FVAFV DK(C)
+ Methionine Sulfoxide
1893.9294 -10.63 508 523 (R)RPCFS ALTPD ETYVP K(A)

+ Cysteine acrylamide

9

NCF1_BOVIN

10

Q95L50

Type IX collagen alpha 1 chain

Molecular weight: 20907

Matches: 5

MOWSE Score: 3.7725965e+003

Likelihood: 5.46e+003

Coverage: 28.34 %

Matching peptides:

MW Delta Start End Sequence

933.4668 -63.69 61 68 (K)LGNNV DFR(I)

1051.6390 -43.95 173 181 (R)IESLP IKPR(G)

2183.0687 42.58 73 90 (R)HLYPN GLPEE YSFLT TFR(M)

832.4476 -30.07 155 161 (K)IMIGV ER(S)

+ Methionine Sulfoxide

1282.5976 -22.54 162 172 (R)SSATL FVDCN R(I)

+ Cysteine acrylamide

11

ALBU_BOVIN

Bovine Serum Albumin

Molecular weight: 69294

Matches: 5

MOWSE Score: 1.1175735e+003

Likelihood: 7.26e+002

Coverage: 10.21 %

Matching peptides:

MW Delta Start End Sequence

926.4862 -156.39 161 167 (K)YLYEI AR(R)

1282.7033 -36.27 361 371 (R)HPEYA VSVLL R(L)

1566.7354 -84.65 347 359 (K)DAFLG SFLYE YSR(R)

* 1887.9876 -6.32 89 105 (K)SLHTL FGDEL CKVAS LR(E)

1887.9195 -42.39 169 183 (R)HPYFY APELL YYANK(Y)

1790.7021 -70.93 267 280 (K)ECCHG DLLEC ADDR(A)
+ Cysteine acrylamide
+ Cysteine acrylamide
+ Cysteine acrylamide
12
ALBU_BOVIN
Bovine Serum Albumin
Molecular weight: 69294
Matches: 16
MOWSE Score: 3.5119435e+010
Likelihood: 1.04e+004
Coverage: 28.17 %
Matching peptides:
MW Delta Start End Sequence
926.4862 -106.87 161 167 (K)YLYEI AR(R)
1282.7033 28.51 361 371 (R)HPEYA VSVLL R(L)
1304.7088 -33.76 402 412 (K)HLVDE PQNLI K(Q)
1478.7881 -58.18 421 433 (K)LGEYG FQNAL IVR(Y)
1510.8355 -34.08 438 451 (K)VPQVS TPTLV EVSR(S)
* 1638.9304 -30.89 437 451 (R)KVPQV STPTL VEVSR(S)
* 1737.8032 -39.00 387 401 (K)DDPHA CYSTV FDKLK(H)
1120.5223 -76.32 588 597 (K)EACFA VEGPK(L)
+ Cysteine acrylamide
1165.5220 -96.70 499 507 (K)CCTES LVNR(R)
+ Cysteine acrylamide
+ Cysteine acrylamide
1193.5169 -102.89 460 468 (R)CCTKP ESER(M)
+ Cysteine acrylamide
+ Cysteine acrylamide
1414.6802 16.07 569 580 (K)TVMEN FVAFV DK(C)
+ Methionine Sulfoxide
1567.6613 9.82 387 399 (K)DDPHA CYSTV FDK(L)
+ Cysteine acrylamide
1589.7759 24.36 139 151 (K)LKPDP NTLCD EFK(A)
+ Cysteine acrylamide

Page 6
1753.8379 17.95 469 482 (R)MPCTE DYLSL ILNR(L)
+ Methionine Sulfoxide
+ Cysteine acrylamide
1893.9294 -3.13 508 523 (R)RPCFS ALTPD ETYVP K(A)
+ Cysteine acrylamide
1920.9291 10.74 529 544 (K)LFTFH ADICT LPDTE K(Q)
+ Cysteine acrylamide
13
Q95L50
Type IX collagen alpha 1 chain
Molecular weight: 20907
Matches: 9
MOWSE Score: 1.6928014e+007
Likelihood: 1.54e+004
Coverage: 62.03 %
Matching peptides:
MW Delta Start End Sequence
816.4018 -95.42 124 130 (K)SVSFS YK(G)
816.4527 -32.98 155 161 (K)IMIGV ER(S)
933.4668 -51.91 61 68 (K)LGNNV DFR(I)
1051.6390 -49.75 173 181 (R)IESLP IKPR(G)
1570.7528 -9.01 99 111 (K)HWSIW QIQDS SGK(E)
2148.0739 -6.99 21 39 (R)IGQDD LPGFD LISQF QIDK(A)
2183.0687 -12.48 73 90 (R)HLYPN GLPEE YSFLT TFR(M)
2618.2765 -0.04 131 154 (K)GLDGS LQTAA FSNLP SLFDS QWHK(I)
832.4476 -3.52 155 161 (K)IMIGV ER(S)
+ Methionine Sulfoxide
1282.5976 -44.29 162 172 (R)SSATL FVDCN R(I)
+ Cysteine acrylamide
14
Q95L50
Type IX collagen alpha 1 chain
Molecular weight: 20907
Matches: 9
MOWSE Score: 1.6928014e+00

Likelihood: 8.48e+003
Coverage: 62.03 %
Matching peptides:
MW Delta Start End Sequence
816.4018 -156.04 124 130 (K)SVSFS YK(G)
816.4527 -93.60 155 161 (K)IMIGV ER(S)
933.4668 -138.67 61 68 (K)LGNNV DFR(I)
1051.6390 -126.57 173 181 (R)IESLP IKPR(G)
1570.7528 -78.20 99 111 (K)HWSIW QIQDS SGK(E)
2148.0739 -53.45 21 39 (R)IGQDD LPGFD LISQF QIDK(A)
Page 7
2183.0687 -61.45 73 90 (R)HLYPN GLPEE YSFLT TFR(M)
2618.2765 -0.10 131 154 (K)GLDGS LQTAA FSNLP SLFDS QWHK(I)
832.4476 -105.98 155 161 (K)IMIGV ER(S)
+ Methionine Sulfoxide
1282.5976 -119.59 162 172 (R)SSATL FVDCN R(I)
+ Cysteine acrylamide
15
Q95L50
Type IX collagen alpha 1 chain
Molecular weight: 20907
Matches: 7
MOWSE Score: 5.0424749e+005
Likelihood: 8.67e+003
Coverage: 48.13 %
Matching peptides:
MW Delta Start End Sequence
816.4018 103.26 124 130 (K)SVSFS YK(G)
816.4527 165.71 155 161 (K)IMIGV ER(S)
933.4668 13.11 61 68 (K)LGNNV DFR(I)
1051.6390 5.50 173 181 (R)IESLP IKPR(G)
1570.7528 -0.05 99 111 (K)HWSIW QIQDS SGK(E)
2183.0687 -17.84 73 90 (R)HLYPN GLPEE YSFLT TFR(M)
2618.2765 0.11 131 154 (K)GLDGS LQTAA FSNLP SLFDS QWHK(I)
1282.5976 -29.95 162 172 (R)SSATL FVDCN R(I)
+ Cysteine acrylamide

16
Q95L50
Type IX collagen alpha 1 chain
Molecular weight: 20907
Matches: 5
MOWSE Score: 1.6468835e+004
Likelihood: 8.25e+003
Coverage: 34.76 %
Matching peptides:
MW Delta Start End Sequence
933.4668 35.82 61 68 (K)LGNNV DFR(I)
1051.6390 30.60 173 181 (R)IESLP IKPR(G)
2148.0739 0.08 21 39 (R)IGQDD LPGFD LISQF QIDK(A)
2183.0687 -1.95 73 90 (R)HLYPN GLPEE YSFLT TFR(M)
1282.5976 -7.80 162 172 (R)SSATL FVDCN R(I)
+ Cysteine acrylamide
Page 8
17
Q95L50
Type IX collagen alpha 1 chain
Molecular weight: 20907
Matches: 8
MOWSE Score: 9.0085540e+005
Likelihood: 5.86e+003
Coverage: 49.20 %
Matching peptides:
MW Delta Start End Sequence
816.4018 -137.06 124 130 (K)SVSFS YK(G)
816.4527 -74.62 155 161 (K)IMIGV ER(S)
933.4668 -125.07 61 68 (K)LGNNV DFR(I)
1051.6390 -117.92 173 181 (R)IESLP IKPR(G)
1570.7528 -50.26 99 111 (K)HWSIW QIQDS SGK(E)
2148.0739 -4.71 21 39 (R)IGQDD LPGFD LISQF QIDK(A)
2183.0687 -24.99 73 90 (R)HLYPN GLPEE YSFLT TFR(M)
832.4476 -86.04 155 161 (K)IMIGV ER(S)
+ Methionine Sulfoxide

1282.5976 -97.22 162 172 (R)SSATL FVDCN R(I)
+ Cysteine acrylamide
18
Q95L50
Type IX collagen alpha 1 chain
Molecular weight: 20907
Matches: 5
MOWSE Score: 1.6468835e+004
Likelihood: 2.56e+003
Coverage: 34.76 %
Matching peptides:
MW Delta Start End Sequence
933.4668 -122.28 61 68 (K)LGNNV DFR(I)
1051.6390 -107.17 173 181 (R)IESLP IKPR(G)
2148.0739 0.02 21 39 (R)IGQDD LPGFD LISQF QIDK(A)
2183.0687 -6.21 73 90 (R)HLYPN GLPEE YSFLT TFR(M)
1282.5976 -90.21 162 172 (R)SSATL FVDCN R(I)
+ Cysteine acrylamide
19
OBP_BOVIN
Odorant-binding protein
Molecular weight: 18503
Matches: 7
MOWSE Score: 4.8550116e+005
Likelihood: 2.71e+004
Coverage: 50.94 %
Page 9
Matching peptides:
MW Delta Start End Sequence
959.4825 -4.49 30 37 (K)IQENG PFR(T)
993.4655 32.50 42 49 (R)ELVFD DEK(G)
1161.5706 1.66 50 59 (K)GTVDF YFSVK(R)
1207.6085 18.62 19 29 (R)TVYIG STNPE K(I)
1359.7259 -11.18 97 108 (R)THLVA HNINV DK(H)
1788.8067 1.86 145 159 (K)NVVNF LENED HPHPE(-)
1947.8486 2.44 74 90 (K)QDDGT YVADY EGQNV FK(I)

20

Q95L50

Type IX collagen alpha 1 chain

Molecular weight: 20907

Matches: 8

MOWSE Score: 9.0085540e+005

Likelihood: 6.84e+003

Coverage: 49.20 %

Matching peptides:

MW Delta Start End Sequence

816.4018 -121.38 124 130 (K)SVSFS YK(G)

816.4527 -58.95 155 161 (K)IMIGV ER(S)

933.4668 -106.00 61 68 (K)LGNNV DFR(I)

1051.6390 -108.98 173 181 (R)IESLP IKPR(G)

1570.7528 -54.97 99 111 (K)HWSIW QIQDS SGK(E)

2148.0739 -18.35 21 39 (R)IGQDD LPGFD LISQF QIDK(A)

2183.0687 -25.77 73 90 (R)HLYPN GLPEE YSFLT TFR(M)

832.4476 -81.84 155 161 (K)IMIGV ER(S)

+ Methionine Sulfoxide

1282.5976 -93.79 162 172 (R)SSATL FVDCN R(I)

+ Cysteine acrylamide

21

Q95L50

Type IX collagen alpha 1 chain

Molecular weight: 20907

Matches: 8

MOWSE Score: 9.0085540e+005

Likelihood: 6.26e+003

Coverage: 49.20 %

Matching peptides:

MW Delta Start End Sequence

816.4018 -133.87 124 130 (K)SVSFS YK(G)

816.4527 -71.44 155 161 (K)IMIGV ER(S)

933.4668 -113.93 61 68 (K)LGNNV DFR(I)

1051.6390 -115.35 173 181 (R)IESLP IKPR(G)

1570.7528 -60.83 99 111 (K)HWSIW QIQDS SGK(E)

2148.0739 -9.04 21 39 (R)IGQDD LPGFD LISQF QIDK(A)
2183.0687 -23.66 73 90 (R)HLYPN GLPEE YSFLT TFR(M)
Page 10
832.4476 -68.62 155 161 (K)IMIGV ER(S)
+ Methionine Sulfoxide
1282.5976 -93.25 162 172 (R)SSATL FVDCN R(I)
+ Cysteine acrylamide

FIGURE 6

Physical Characteristics of Protein fragments found in Calcium Peptacan after ion-exchange treatment and 2D electrophoresis.

Fragment ID#	Estimated isoelectric point	Estimated Molecular Weight
1	4.0	74kDa
2	4,1	65kDa
3	3.9	50kDa
	(this band has now been shown to correspond to fragments from 2 or 3 proteins identified as bovine COMP and either bovine alpha-1 antiprotease inhibitor or Endopin-1)	
4	3.7	45kDa
5	3.4	40kDa
6	4.6	50kDa
7	6.3	67kDa
8	6.1	65kDa
9	6.3	60kDa
10	6.3	38kDa
11	6.0	30kDa
12	6.4	25kDa
13	6.8	22kDa
14	7.8	20kDa
15	8.2	18kDa
16	8.3	19kDa
17	8.6	20kDa
18	9.1	19kDa
19	5.3	12kDa
20	6.2	22kDa
21	7.2	21kDa

(i) Cartilage oligomeric matrix protein [Fragment] - bovine

```
          10          20          30          40          50          60
          |           |           |           |           |           |
DGVLNEKDNC PLVRNPDQRN TDGDKWGDAC DNCRSQKNDD QKDTDKDGRG DACDDDIDGD

          70          80          90          100         110         120
          |           |           |           |           |           |
RIRNPVDNCP KVPNSDQKDT DGDGVGDACD NCPQKSNADQ RDVDHDFVGD ACDSDQDQDG

          130         140         150         160         170         180
          |           |           |           |           |           |
DGHQDSKDNC PTVPNSAQQD SDHDGQGDAC DDDDDNDGVP DSRDNCRLVP NPGQEDMDRD

          190         200         210         220         230         240
          |           |           |           |           |           |
GVGDACQGDF DADKVVDKID VCPENAEVTL TDFRAFQTVV LDPEGDAQID PNWVVLNQGM

          250         260         270         280         290         300
          |           |           |           |           |           |
EIVQTMNSDP GLCVGYTAFN GVDFEGPFHV NTATDDDYAG FIFGYHHSSS FYVVMWKQME

          310         320         330         340         350         360
          |           |           |           |           |           |
QTYWQANPFR AVAEPGIQLK AVKSSTGPGE QLRNALWHTG DTASQVRLLW KDPRNVGWKD

          370         380         390         400         410         420
          |           |           |           |           |           |
KTSYRWFLQH RPQVGYIRVR FYEGPELVAD SNVILDTTMR GGRLGVFCFS QENIIWANLR

          430
          |
YRCNDTIPED YEAQRLLQA
```

(ii) Odorant-binding protein - bovine

```
          10          20          30          40          50          60
          |           |           |           |           |           |
AQEEEAEQNL SELSGPWRTV YIGSTNPEKI QENGPFRTYF RELVFDDEKG TVDFYFSVKR

          70          80          90          100         110         120
          |           |           |           |           |           |
DGKWKNVHVK ATKQDDGTYV ADYEGQNVFK IVSLSRTHLV AHNINVDKHG QTTELTELFV

          130         140         150
          |           |           |
KLNVEDEDLE KFWKLTEDKG IDKKNVVNFL ENEDHPHPE
```

Figure 7

Shows the partial amino acid sequence of the bovine NC4 domain of type IX collagen alpha 1 chain sequence obtained from the ExPASy TrEMBL database on which the peptide sequences obtained from the MALD-MS peptide mass fingerprinting of gel spot 13 (Figure 4) has been superimposed as bolded type where they are identical.

```
        10        20        30        40        50        60
        |         |         |         |         |         |
PRFPVNSNSN GENELCPKVR IGQDDLPGFD LISQFQIDKA ASRRAIQRVV GSTALQVAYK

        70        80        90        100       110       120
        |         |         |         |         |         |
LGNNVDFRIP TRHLYPNGLP EEYSFLTTFR MTGSTLEKHW SIWQIQDSSG KEQVGVKING

        130       140       150       160       170       180
        |         |         |         |         |         |
QTKSVSFSYK GLDGSLQTAA FSNLPSLFDS QWHKIMIGVE RSSATLFVDC NRIESLPIKP

RGQIDVD
```

Figure 9:
Shows the amino acid sequence of the NC4 domain of the type IX collagen alpha 1 chain from 17 day old chick embryos sterna as reported by Vasios et al. (J Biological Chem. 263, 2324-2329, 1998) on which the amino acid sequences identified from the MALDI-MS analysis of the polypeptides separated by 2D electrophoresis have been superimposed indicating where the sequences are identical as bold type and underlined.

PRO-**ARG**-PHE-**PRO-VAL**-ASN-SER-ASN-SER-ASN / GLY-GLU-ASN-GLU-

LEU-CYS-PRO-LYS-VAL-**ARG / ILE-GLY-GLN-ASP-ASP-LEU-PRO-GLY-**

PHE-ASP / LEU-ILE-SER-GLN-PHE-GLN-ILE-ASP-**LYS-ALA / ALA**-SER-

ARG-ARG-ALA-ILE-**GLN-ARG-VAL-VAL / GLY-SER-THR-ALA-LEU**-GLN-

VAL-ALA-TRY-LYS / LEU-GLY-ASN-ASN-VAL-ASP-PHE-ARG / **THR**-ARG-

HIS-LEU-**TYR**-PRO-**ASN-GLY-LEU-PRO** / GLU-**GLU-TYR-SER-PHE-LEU-**

THR-THR-PHE-ARG / MET-THR-GLY-SER-**THR-LEU**-GLY-**LYS**-HIS-**TRP** /

SER-**ILE-TRP-GLN-ILE-GLN-ASP-SER-SER-GLY / LYS-GLU-GLN-VAL-**

GLY-VAL-LYS-ILE-**ASN-GLY** / GLN-THR-**LYS-SER-VAL**-SER-**PHE-SER-**

TRY-**LYS / GLY**-LEU-**ASP-GLY-SER-LEU-GLN-THR-ALA**-ALA / **PHE**-SER-

ASN-**LEU-PRO**-SER-**LEU-PHE-ASP-SER / GLN-TRP-HIS-LYS-ILE-MET-**

ILE-GLY-**VAL-GLU** / ARG-SER-**SER**-ALA-**THR-LEU-PHE**-VAL-**ASP-CYS** /

ASN-ARG-ILE-**GLU**-SER-**LEU**-PRO-**ILE-LYS-PRO**

Figure 10:

Shows the human NC4 domain of the type IX collagen alpha 1 chain (sequences 24-268) obtained from the Swiss-Prot & TrEMBL data-bases (released 07-June-2004) on which the amino acid sequences identified from the MALDI-MS analysis of the polypeptides separated by 2D electrophoresis have been superimposed showing where the sequences are identical as bold type and underlined. Sequence 1-23 is the signal sequence for the human NC4 domain of the type IX collagen alpha 1 chain.

1 *Met Lys Thr Cys Trp Lys Ile Pro Val Phe Phe Phe Val Cys Ser* **16** *Phe Leu Glu Pro Trp Ala Ser Ala* **23** Ala Val Lys Arg Arg **Pro Arg 31 Phe Pro Val Asn Ser Asn Ser Asn Gly** Gly **Asn Glu Leu Cys Pro 46 Lys** Ile **Arg Ile Gly Gln Asp Asp Leu Pro Gly Phe Asp Leu Ile 61 Ser Gln Phe Gln** Val **Asp Lys Ala Ala Ser Arg Arg Ala Ile Gln 76 Arg Val Val Gly Ser** Ala Thr **Leu Gln Val Ala Tyr Lys Leu Gly 91 Asn Asn Val Asp Phe Arg** Ile Pro Thr Arg Asn Leu Tyr Pro Ser **106** Gly Leu **Pro Glu Glu Tyr Ser Phe Leu Thr Thr Phe Arg Met Thr 121 Gly Ser Thr Leu** Lys **Lys** Asn **Trp** Asn **Ile Trp Gln Ile Gln Asp 136 Ser Ser Gly Lys Glu Gln Val Gly Ile Lys Ile Asn Gly Gln Thr 151** Gln **Ser Val** Val **Phe Ser Tyr Lys Gly Leu Asp Gly Ser Leu Gln 166 Thr Ala Ala Phe Ser Asn Leu** Ser **Ser Leu Phe Asp Ser Gln Trp 181 His Lys Ile Met Ile Gly Val Glu Arg Ser Ser Ala Thr Leu Phe 196 Val Asp Cys Asn Arg Ile Glu Ser Leu Pro Ile Lys Pro** Arg Gly **211** Pro Ile Asp Ile Asp Gly Phe Ala Val Leu Gly Lys Leu Ala Asp **226** Asn Pro Gln Val Ser Val Pro Phe Glu Leu Gln Trp Met Leu Ile **241** His Cys Asp Pro Leu Arg Pro Arg Arg Glu Thr Cys His Glu Leu **256** Pro Ala Arg Ile Thr Pro Ser Gln Thr Thr Asp Glu Arg **268**

Figure 11

Protocols used to evaluate polypeptides (PPs) and drugs in the rat CIA model

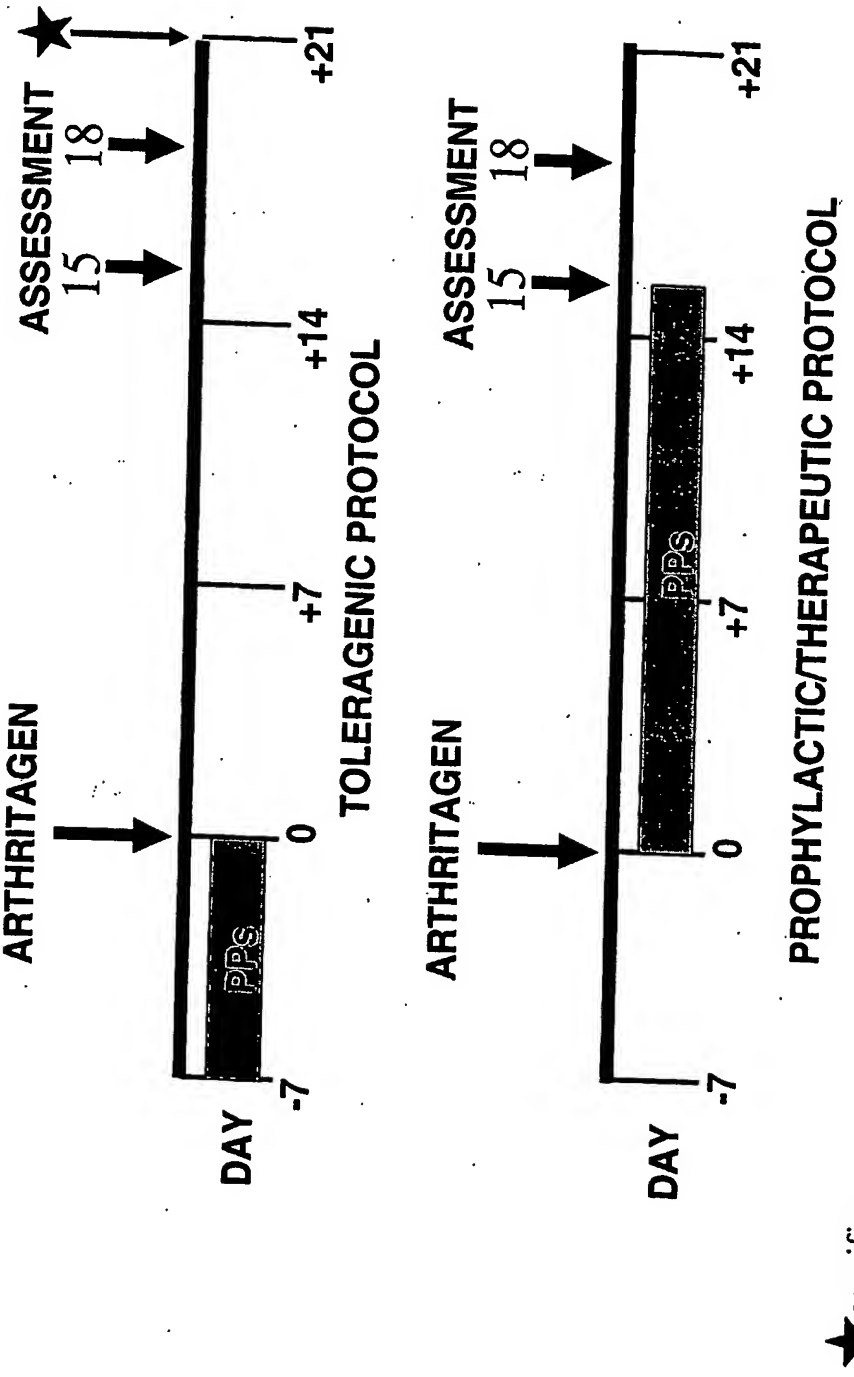


Figure 12

Results demonstrating anti-arthritic activity of the polypeptides INR-195 and INR-126 relative to ATM in the Rat CIA model using the Prophylactic/Therapeutic protocol(15 days treatment)

		Mean arthritis scores			Signs of arthritis Day 15			Signs of arthritis Day 18		
Rx n=4	Dose mg/kg	Day 13	Day 15	Day 18	R/paw swell (mm)	F/paw swell (mm)	Weight change (grams)	R/paw swell (mm)	F/paw swell (mm)	Weight change (grams)
None	___	0.7	1.5	1.8	0.7	2.1	+40	0.8	2.0	-04
INR-126	20 (oral)	0.2**	0.8*	1.3	0.4	1.3*	+47	0.7	2.0	+06
INR-126	200 (oral)	0.2**	0.5*	1.7	0.1	0.1**	+58	0.8	2.3	+04
INR-195	20 (oral)	0.2*	0.7*	1.8	0,2	0.1**	+45	0.7	2.0	+09
ATM	6.3 (SC)	0.8	2.1	2.1	0.7	2.5	+47	0.8	3.2	+04

ATM = Aurothiomalate, SC = subcutaneously, ** = $p < 0.005$ * = $p < 0.05$ relative to none

Figure 13

Results demonstrating the anti-arthritic activity of the polypeptides INR-195 and INR-126 in the Rat CIA model using the toleragenic protocol where preparations are given for 7 days before inducing arthritis

		Mean arthritis scores			Signs of arthritis Day 15			Signs of arthritis Day 18		
Rx n=4	Dose mg/kg	Day 13	Day 15	Day 18	R/paw swell (mm)	F/paw swell (mm)	Weight change (grams)	R/paw swell (mm)	F/paw swell (mm)	Weight change (grams)
None	___	0.7	1.3	1.5	0.8	2.8	+58	0.8	2.5	-01
INR-126	20 (oral)	0.1**	0.6*	0.5**	0.2*	1.4*	+50	0.06**	1.1*	+09
INR-195	20 (oral)	0.6	0.5*	1.5	0.1*	0.1*	+44	0.6	1,5	+05

** = $p < 0.005$ * = $p < 0.05$ relative to no treatment group

FIGURE 14

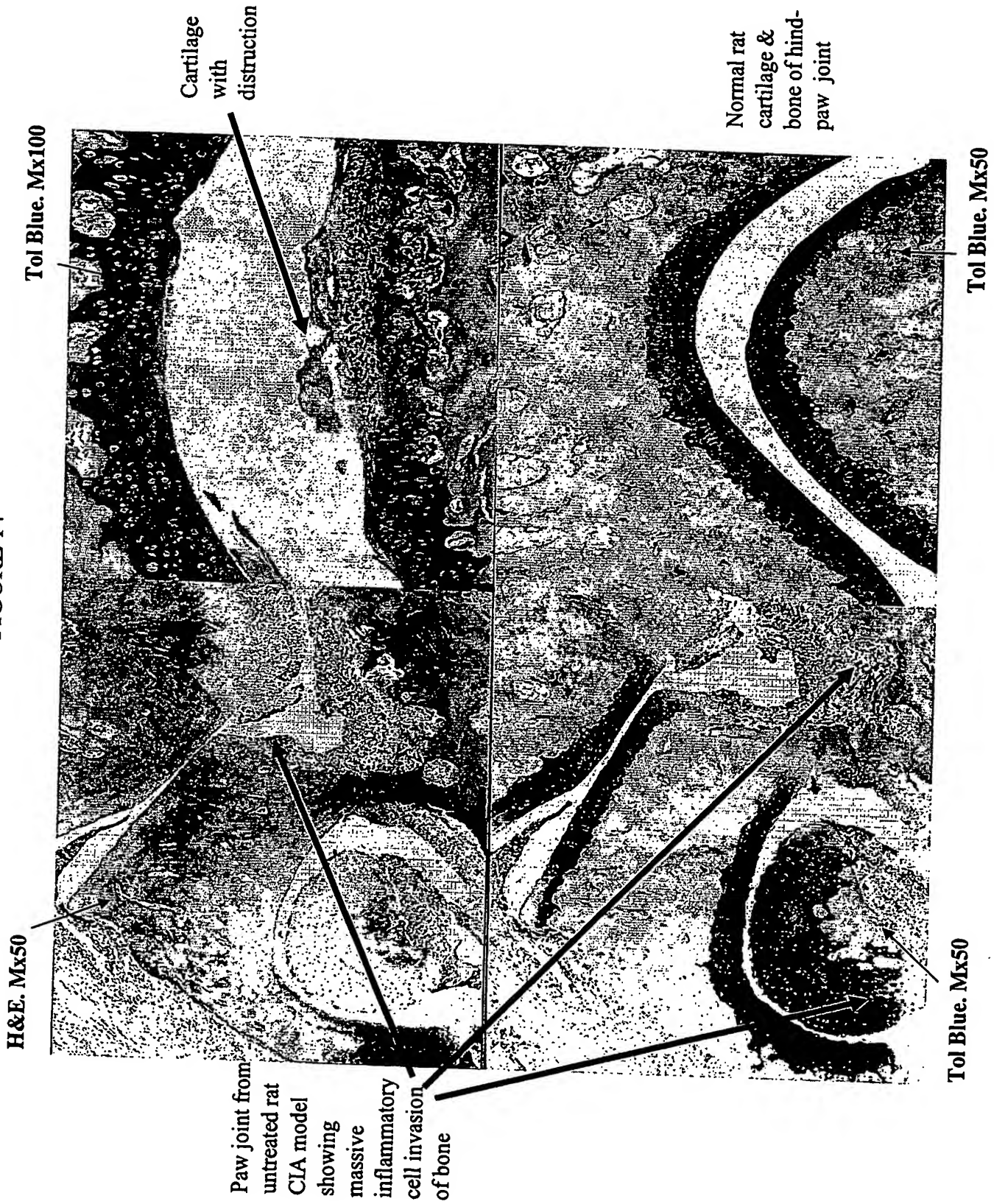
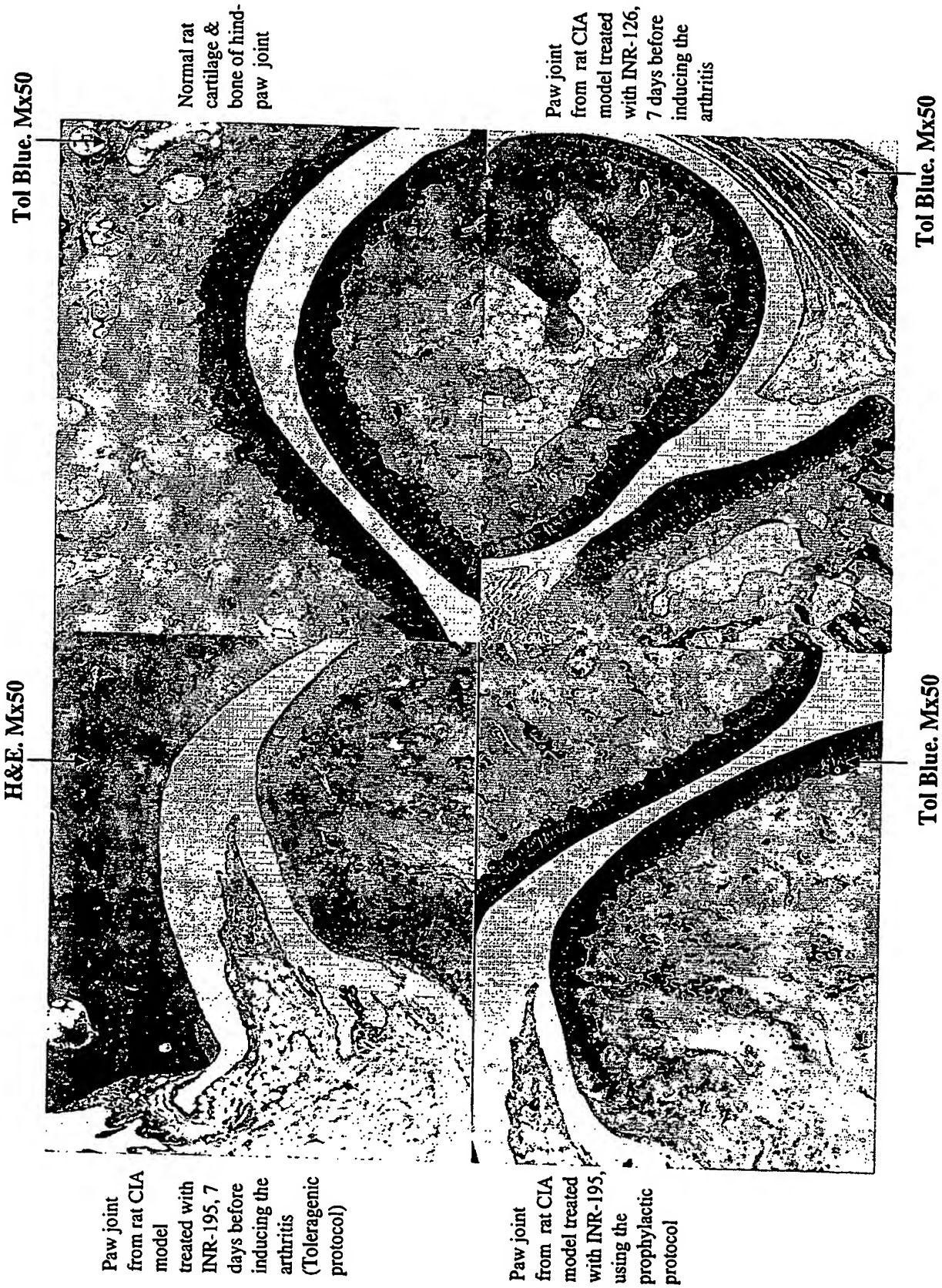

H&E. Mx50
Tol Blue. Mx100
Cartilage with distruction
Normal rat cartilage & bone of hind-paw joint
Tol Blue. Mx50
Paw joint from untreated rat CIA model showing massive inflammatory cell invasion of bone
Tol Blue. Mx50

FIGURE 15


H&E. Mx50
Tol Blue. Mx50
Normal rat cartilage & bone of hind-paw joint
Paw joint from rat CIA model treated with INR-126, 7 days before inducing the arthritis
Tol Blue. Mx50
Paw joint from rat CIA model treated with INR-195, 7 days before inducing the arthritis (Toleragenic protocol)
Tol Blue. Mx50
Paw joint from rat CIA model treated with INR-195, using the prophylactic protocol

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☑ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.